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Fumigant Toxicity of Essential Oils of Nine Plant Species from Asteraceae and Clusiaceae against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

Saban Kordali*; Erol Yildirim*; Gulten Yazici*; Bugrahan Emsen; Gulbahar Kabaagac* and Sezai Ercisli*****

*Ataturk University, Faculty of Agriculture, Department of Plant Protection, 25240, Erzurum- Turkey
**Karamanoğlu Mehmetbey University, Kamil Özdağ Faculty of Science, Department of Biology, 70200, Karaman-TURKEY
***Ataturk University, Faculty of Agriculture, Department of Horticulture, 25240, Erzurum- Turkey

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ABSTRACT

Essential oils obtained from nine different plant species (*Achillea coarctata* Poir., Achillea *gypsicola* Hub.-Mor., Artemisia dracunculus L., Artemisia vulgaris L., *Helichrysum plicatum* Dc., *Tanacetum agrophyllum* (L.), *Taraxacum officinale* (L.) (Asteraceae), *Hypericum scabrum* L. and Hypericum *perforatum* L. (Clusiaceae)) were tested against adults of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Results clarified that essential oils of *A. coarctata*, *A. gypsicola*, *T. agrophyllum*, *H. scabrum* and H. *perforatum* had highest insecticidal effects on *S. granarius* adults, compared with the control. Mortality rate of *S. granarius* adults increased significantly ($p<0.01$), as the dosage level and/or exposure time increased. Treatments with the essential oils of *A. coarctata*, *A. gypsicola*, *A. dracunculus*, *T. agrophyllum*, *H. scabrum* and H. *perforatum* showed high levels of mortalities in *S. granarius* adults, when they were applied at the higher tested concentrations (10 & 20 µl) at all post treatment periods or at a moderate concentration (5µl) with a long exposure period (48 or 96 h). The essential oils of A. vulgaris, *H. plicatum* and *T. officinale* had either very low or no effects. Mortality percentages of *S. granarius* adults, after 96 h of exposure at the maximum dose (20 µl essential oil) of *A. coarctata*, *A. gypsicola*, A. dracunculus, *H. scabrum*, H. *perforatum*, *T. agrophyllum*, *H. plicatum* A. vulgaris and *T. officinale* attained 100, 100, 100, 100, 100, 98.99, 83.84, 23.23, and 18.18%, respectively. No mortality was recorded in the control. After 96 h of treatment, highest levels of mortalities (95.96 %) were recorded at the dose of 1 µl essential oil of H. *perforatum*. They were (84-100 %) at the dose of 5µl of the essential oils of *A. coarctata, A. gypsicola*, A. dracunculus, *T. agrophyllum* and H. *perforatum*, (93.94 %) at the dose of 10 µl for *H. scabrum* and (83.84 %) at the dose of 20 µl for *H. plicatum.*

Key words: *Sitophilus granarius*, Asteraceae, Clusiaceae, Essential Oils, Fumigant toxicity.

INTRODUCTION

The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) is a well-known pest causing economically yield losses in stored products in Turkey and many other countries (Yildirim *et al*., 2005a). Nowadays, insect control strategy in stored-food products relies heavily upon the use of gaseous fumigants and residual insecticides, both of which pose serious hazards to warm-blooded animals and the environment. Fumigation is still one of the most effective methods for the prevention of storage losses. However, the availability of fumigants for insect control has dwindled drastically lately. As of now, only one fumigant is still in use, namely, phosphine. The former is suspected of leaving residues that are harmful to mammals, while the latter, which is in wide use, has shown alarming indications of development of insect resistance (Yildirim *et al.,* 2001). Thus, it would be of marked benefit for the preservation of stored products to uncover and develop new compounds that have the potential to replace the toxic fumigants, less harmful, yet easy, simple, and convenient to use.

Integrated Pest Management (IPM) has to face up to the economic and ecological consequences of the use of pest control measures. Sixty years of sustained struggle against harmful insects using synthetic and oil-derivative molecules have produced perverse secondary effects. The diversification of the approaches inherent in IPM is necessary for better environmental protection. Among the alternative strategies, the use of different insecticidal allelochemicals of plants appears to be promising. Aromatic plant derivatives and plant oils are among the most efficient botanical substances. Their activities are manifold. They induce fumigant and topical toxicity as well as antifeedant and repellent effects (Regnault, 1997). Essential oils are presently regarded as a new class of ecological products for controlling insect pests. They are among the best-known substances tested against some insect pests. Moreover, they are potential and safe sources of alternative compounds to currently used fumigants, as they have low toxicity to warm-blooded animals, high volatility, and toxicity to stored grain insect pests (Shaaya *et al.*, 1997). Contact and fumigant insecticidal actions of plant essential oils have been well demonstrated against stored product pests (Huang *et al.*, 1997; Ho *et al.*, 1997; Tripathi *et al.*, 2000; Papachristos and Stamopoulos, 2002; Lee *et al.*, 2003; Yildirim *et al.*, 2005 a, b; Tapondjou *et al.*, 2005 and

Kordali *et al*., 2006).

Aim of the present study was to estimate fumigant toxicity of some hydrodistilled essential oils against *S. granarius*.

MATERIALS AND METHODS

Source and rearing of the test insect

S. granarius was obtained from Erzurum storage house, Turkey. Wheat grains were purchased from local market and stored in a freezer at -20 °C. The wheat was washed by tap water, dried and heated to prevent pre-infestation. *S. granarius* adults were reared in the laboratory at 25±1 °C, 64±5% RH and L: D = 12:12 at the Department of Plant Protection, Ataturk University, Turkey. Obtained adults from the laboratory culture were stored in separate insect cages provided with wheat. Tests were also carried out under the same laboratory conditions.

Determination of adults' age

Four to six day-old *S. granarius* adults were used as a test insect. In order to get adults at the same age, few grains of wheat that included larvae and pupae were placed separately in Petri dishes. After adult emergence, adults of the same age were collected daily and used.

Isolation of essential oils

Achillea coarctata Poir., *A. gypsicola* Hub.-Mor., Artemisia vulgaris L., *Helichrysum plicatum* Dc., *Tanacetum agrophyllum* (L.), *Taraxacum officinale* (L.) (Asteraceae), *Hypericum scabrum* L. and H. *perforatum* L. (Clusiaceae) were collected at the flowering stage in August 2009 in Turkey. Separately, the aerial parts of Artemisia dracunculus L. were purchased at vegetative stage from the local market. Collected plant materials were dried in shadow and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation (plant material in boiling water) using a Clevenger-type apparatus for 4 hours. Hydrodistillation of *A. coarctata*, *A. gypsicola,* A. dracunculus, A. vulgaris, *H. plicatum*, *T. agrophyllum*, *T. officinale*, *H. scabrum* and H. *perforatum* yielded 0.21, 0.65, 1.00, 0.78, 0.82, 0.19, 0.75, 0.17 and 0.24 % (w/w) of essential oils, respectively. The yields were based on dry materials of plant samples.

Bioassays

In order to test the toxicity of the essential oils against *S. granarius* adults, 33 individuals with 33 grains of wheat were placed into Petri dishes (9 cm diameter). 1, 5, 10 and 20 micro liters of the oils were applied with an automatic pipette on a filter paper (2 × 2 cm) attached to the upside of the Petri dishes, corresponding to 7.70, 38.47, 76.91 and 153.84 µl/l air concentrations. Mortality rate of the adults was determined after an exposure for 24, 48 and 96 h. Petri dishes, applied with only sterile water served as control. Three replicates were used/dose/exposure time. Insecticidal action of oils was expressed as % mean mortality of the adults.

Statistical analysis

Differences among the fumigant toxicities of the essential oils tested were determined according to analysis of variance (ANOVA) test by using SPSS 15.0 software package. Differences between means were tested through Duncan tests and values with $p<0.01$ were considered significantly different. LD_{25}, LD_{50} and LD_{90} values at 96 h were calculated by SPSS. Probit analysis of concentration-mortality data was conducted to estimate the LD_{25}, LD_{50} and LD_{90} values and associated 95 % confidence limits for each treatment (EPA Probit Analysis).

RESULTS AND DISCUSSION

Toxicity effects of the essential oils extracted from the nine tested plant species; *A. coarctata*, *A. gypsicola*, A. dracunculus, A. vulgaris, *H. plicatum*, *T. agrophyllum*, *T. officinale*, *H. scabrum* and H. *perforatum* on adults of *S. granarius* were estimated. The results showed that the essential oils of *A. coarctata, A. gypsicola*, *T. agrophyllum*, *H. scabrum* and H. *perforatum* had insecticidal effects on *S. granarius* adults in comparison with the control (Table 1).

Analysis of variance demonstrated that the effects of these essential oils on the mortality rate of *S. granarius* was highly significant on the basis of both dosage rate and exposure time ($p<0.01$). Higher doses and longer exposure times scored maximum toxicities on *S. granarius*. Treatments with the essential oils of *A. coarctata*, *A. gypsicola*, *T. agrophyllum*, *H. scabrum* and H. *perforatum* showed highest mortality rates, while low mortality rate was found in case of A. dracunculus and negligible effects existed, when the essential oils of A. vulgaris, *H. plicatum* and *T. officinale* were tested (Table 1). On the other hand, mortality percentages, after 96 h of treatments with the maximum dose (20 µl) of the essential oils of *A. coarctata*, *A. gypsicola*, A. dracunculus, *H. scabrum*, H. *perforatum, T. agrophyllum*, *H. plicatum* A. vulgaris and *T. officinale* attained 100, 100, 100, 100, 100, 98.99, 83.84, 23.23, and 18.18 %, respectively (Table 1). No mortality was recorded in the control.

As shown also in table (1), highest mortality percentages of the essential oils were achieved 96 h post treatments, at the dose of both 10 and 20 µl of

Table (1): Percent mortality of the essential oils of nine plant species to the adults of *Sitophilus granarius* (L.) under laboratory conditions

Doses (µl)	Exposure Time (h)	*A.coarctata* M	*A.gypsicola* M	*A.dracunculus* M	*A.vulgaris* M	*H.plicatum* M	*T.agrophyllum* M	*T.officinale* M	*H.scabrum* M	*H.perforatum* M
Control	24	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
	48	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
	96	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
	M	$0.00a_1$	$0.00a_1$	$0.00a_1$	$0.00a_1$	$0.00a_1$	$0.00a_1$	$0.00a_1$	0.00a	$0.00a_1$
1	24	1.01a	12.12ab	2.02a	0.00a	0.00a	13.13ab	0.00a	7.07a	35.35b
	48	4.04a	25.25bc	27.27b	1.01a	1.01a	28.28bc	1.01a	12.12a	70.71c
	96	20.20b	50.51d	88.89c	15.15b	21.21c	65.66def	16.16b	51.52b	95.96d
	M	$8.42b_1$	$29.29b_1$	$39.39b_1$	$5.39b_1$	$7.41b_1$	$35.69b_1$	$5.72b_1$	$23.57b_1$	$67.34b_1$
5	24	2.02c	32.32c	5.05a	0.00a	0.00a	49.49cd	0.00a	11.11a	56.57c
	48	22.22b	85.86ef	33.33b	2.02a	1.01a	77.78efg	2.02a	14.14a	86.87d
	96	87.88d	100.00f	84.85c	18.18bc	33.33d	94.95g	16.16b	48.48b	100.00d
	M	$37.37c_1$	$72.73c_1$	41.08b	$6.73b_1$	$11.45b_1$	$74.07c_1$	$6.06b_1$	$24.58b_1$	$81.14c_1$
10	24	7.07a	76.77e	6.06a	0.00a	1.01a	58.59de	0.00a	45.45b	62.63c
	48	65.66c	98.99f	82.83c	2.02a	5.05ab	87.88fg	2.02a	62.63b	86.87d
	96	100.00e	100.00f	100.00c	19.19bc	57.58e	97.98g	18.18b	93.94c	100.00d
	M	$57.58d_1$	$91.92d_1$	$62.96c_1$	$7.07b_1$	$21.21c_1$	$81.48c_1d_1$	$6.73b_1$	$67.34c_1$	$83.16c_1$
20	24	10.10a	76.77e	8.08a	0.00a	2.02a	84.85fg	0.00a	100.00c	67.68c
	48	81.82d	100.00f	91.91c	3.03a	11.11b	95.96g	2.02a	100.00c	94.95d
	96	100.00e	100.00f	100.00c	23.23c	83.84f	98.99g	18.18b	100.00c	100.00d
	M	$63.97e_1$	$92.26d_1$	$49.16c_1$	$8.75b_1$	$32.32d_1$	$93.27d_1$	$6.73b_1$	$100.00d_1$	$87.54c_1$

M (Mean of three replicates, each set-up with 33 adults).
a, b, c, d, e, f, g : in the same column the exposure times differ ($p<0.01$).
a_1, b_1, c_1, d_1, e_1 : in the same column the doses differ ($p<0.01$).

Table (2): 96 h LD_{25}, LD_{50} and LD_{90} values (µl) of the essential oils of nine plant species on *Sitophilus granarius* (L.) adults under laboratory conditions

Treatments	LD_{25} (Range)	LD_{50} (Range)	LD_{90} (Range)	Slope (±SE) (Range)
Achillea coarctata	1.153	1.899	4.899	3.114±0.269
	(0.930 - 1.373)	(1.613 - 2.212)	(4.086 - 6.145)	(2.588 - 3.640)
Achillea gypsicola	0.718	0.994	1.843	4.780±2.068
	(0.104 - 0.861)	(0.753 - 1.194)	(1.381 - 52.563)	(0.728 - 8.833)
Artemisia dracunculus	0.007	0.052	2.091	0.798±0.215
	(0.000 - 0.064)	(0.000 - 0.234)	(0.829 - 3.747)	(0.375 - 1.220)
Artemisia vulgaris	*	*	*	*
Helichrysum plicatum	1.834	6.059	58.705	1.299±0.149
	(1.189 - 2.492)	(4.767 - 7.710)	(36.672 - 119.972)	(1.006 - 1.592)
Tanacetum agrophyllum	0.211	0.550	3.408	1.618±0.224
	(0.082 - 0.371)	(0.297 - 0.815)	(2.576 - 4.814)	(1.179 - 2.058)
Taraxacum officinale	*	*	*	*
Hypericum scabrum	0.611	1.767	13.928	1.462±0.160
	(0.346 - 0.901)	(1.266 - 2.284)	(9.827 - 20.241)	(1.149 - 1.775)
Hypericum perforatum	0.103**	0.196**	0.662 **	2.427±2.121**

* : LD values were not calculated due to very high levels   ** : Narrow limits

A. coarctata (100 %), 5, 10 and 20 µl of *A. gypsicola* (100 %), 10 and 20 µl of *A. dracunculus* (100 %), 20 µl of *A. vulgaris* (23.23 %), 20 µl of *H. plicatum* (83.84 %), 10 µl of *T. agrophyllum* (97.98 %), 10 and 20 µl of *T. officinale* (18.18 %), 20 µl of *H. scabrum* (100%) and equal 5, 10 and 20 µl of H. *perforatum* (100 %).

The present results showed that the essential oils of *A. coarctata*, *A. gypsicola*, *T. agrophyllum*, *H. scabrum* and H. *perforatum* erformed varying degrees of insecticidal activity against adults of *S. granaries*, which increased with increasing dose and exposure times.

The most effective one among the nine plant species was H. *perforatum*, according to LD_{25}, LD_{50} and LD_{90} values 0.103, 0.196, and 0.662, respectively at 96 h. *A. gypsicola* ranked second as the 96 h LD_{90} value was 1.843. At the same time, *A. dracunculus* and *T. agrophyllum* had low LD_{90} values (2.091 for *A. dracunculus* and 3.408 for *T. agrophyllum*). *A. vulgaris* and *T. officinale* had the highest LD values (Table 2).

In general, the toxicity of essential oils isolated from plant samples against stored pests was mainly related to their major components (Huang *et al.*, 1997; Ho *et al.*, 1997; Tripathi *et al.*, 2000; Papachristos and Stamopoulos, 2002; Lee *et al.*, 2003; Yildirim *et al.*, 2005a. b; Tapondjou *et al.*, 2005 and Kordali *et al.*, 2006). These results suggested that the essential oils isolated from different plant species might have different toxicity levels, which can be attributed to their different chemical composition and different major and/or minor constituents.

The development of natural or biological insecticides will help to decrease the negative effects (residues, resistance and environmental pollution) of synthetic chemical insecticides. In this respect, bio-insecticides may be also effective, selective, biodegradable and associated with little development of resistance in the pest population and consequently safer to the environment. In this study, 96 h of exposure to the maximum dose (20 µl) of essential oils of *A. coarctata*, *A. gypsicola*, *A.dracunculus*, *T. agrophyllum*, *H. scabrum* or H. *perforatum* caused the highest mortality rates in *S. granarius* adults. These compounds could to be potential insecticidal agents for controlling the adults of *S. granarius* in stored food products. Obtained results and those reported earlier clearly indicated that the variations in the effects of essential oils are regarded to the stage, the species of insect and plant origin of the essential oil. Not all the tested essential oils showed satisfactory effectiveness, but the essential oils of *A. coarctata*, *A. gypsicola*, *A.dracunculus*, *T. agrophyllum*, *H. scabrum* and H. *perforatum* proved to be promising as control alternatives against stored product insects, especially, *S. granarius.* However, further studies are still needed to evaluate the cost, efficacy and safety of the active insecticidal ingredients and essential oils of those plants, on a wide range of pests and beneficial arthropods prevailing in the commercial stores. El-Sisi and Mahgoub (1996) reported that camphor oil had high killing effect against the rice weevil, *Sitophilus oryzae*.

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